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Molecular and Cytogenetic Characterization of a Recurrent Unbalanced Translocation (4;21)(p16.3;q22.1): Relevance to the Wolf-Hirschhorn and Down Syndrome Critical Regions

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We report on an aneuploidy syndrome due to the unbalanced segregation of a familial translocation (4;21)(p16.3;q22.1) causing a partial 4p monosomy and a partial 21q trisomy. The three affected children presented with severe failure to thrive, short stature, microcephaly, profound hypotonia, and mental retardation. The face, very similar in the three children, is characterized by frontal bossing, upslanting of the palpebral fissures, short nose, and deep set ears, giving the overall appearance of the Down syndrome. The molecular study has defined the aneuploid segment on both 4p and 21q. Most of the Down syndrome critical region was found to be trisomic, while only part of the candidate Wolf-Hirschhorn syndrome critical region was deleted, suggesting that this region is not critical for the major malformations characteristic for WHS.

KEY WORDS: Wolf-Hirschhorn syndrome, Down syndrome, FISH, chromosome 4p

INTRODUCTION

The Wolf-Hirschhorn syndrome (WHS) is a multiple congenital anomalies/mental retardation syndrome caused by the deletion of the distal short arm of chromosome 4p. The main clinical findings include pre- and postnatal growth retardation, severe developmental delay, typical facial features and malformations of heart, genitourinary system, and skeletal system [Wilson et al., 1981]. WHS usually results from variable de novo deletions of 4p, though it can be associated with translocations and ring chromosomes involving the 4p region [Lurie et al., 1980]. When due to parental translocation, a preponderance of paternal origin has been observed [Dallapiccola et al., 1994]. Recently, the critical region for WHS (WHSCR) has been restricted to about 2 Mb in the telomeric 4p region [Gandelman et al., 1992; Estabrooks et al., 1994; Zackai et al., 1994].

Down syndrome (DS), the most common chromosomal disorder of man, is caused by trisomy of chromosome 21. DS is characterized by a complex phenotype including characteristic facial and skeletal appearance, mental retardation, and congenital heart disease. Analysis of genotype-phenotype correlations in rare cases with partial trisomy 21q regions has led to the definition of chromosome 21 regions responsible for many of the major clinical traits of DS including mental retardation [Delabar et al., 1993; Korenberg et al., 1994].

Here we report on the familial recurrence of an aneuploidy syndrome due to the unbalanced inheritance of a parental translocation (4;21)(p16.3;q22.1) causing partial 4p monosomy and a partial 21q trisomy. The phenotype is characterized by severe mental and growth retardation and relatively mild physical anomalies, probably resulting from the overlap of WHS and DS traits. The molecular study has defined the aneuploid segments of both 4p and 21q, showing monosomy for only a part of the candidate WHSCR, though most of the typical features of WHS are not present, and partial trisomy 21 extending from 21q22.1 to 21qter.

CLINICAL REPORTS

Patients 1, 2, and 3 belong to the pedigree shown in Figure 1. Clinical findings are summarized in Table I.

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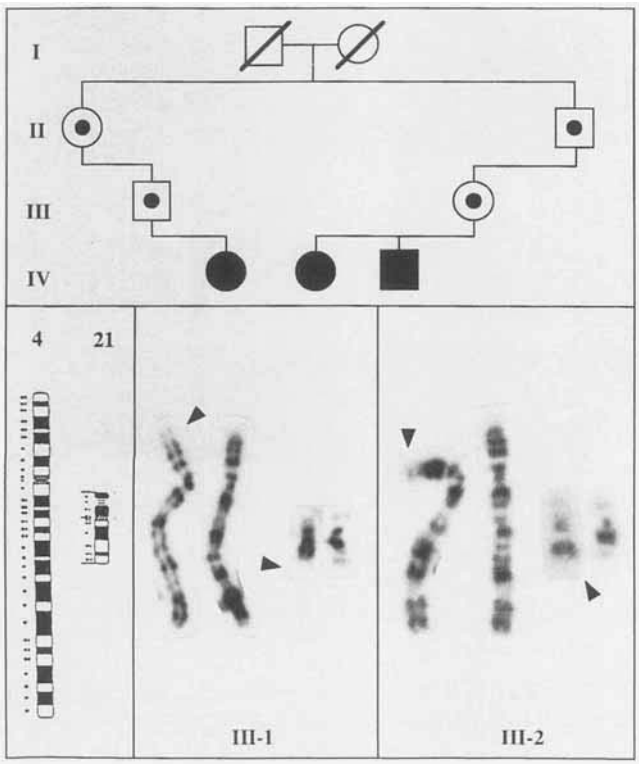

Fig. 1. **Top**, affected (patients 1, 2, and 3, respectively) and carrier members of the pedigree; **bottom**, partial HRG karyotypes of the carrier parents III-1 and III-2.

TABLE I. Clinical Findings Observed in the Three Patients and Their Occurrence in Down Syndrome (DS) and Wolf-Hirschhorn Syndrome (WHS)

	Patients				
	1	2	3	DS	WHS
Low birth weight	+	+	+		+
Failure to thrive	+	+	+	+	+
Severe developmental delay	+	+	+	+	+
Microcephaly	+	+	+	+	+
Low-set ears	+	+	+	+	+
Frontal bossing			+		+
Mid-face hypoplasia	+	+	+	+	
Upslanting palpebral fissures	+	+	+	+	+
Bilateral epicanthal folds	+	+	+	+	+
Blue sclerae	+	+	+		
Brushfield spots	+			+	
Short nose	+	+	+	+	
Micrognathia	+	+			+
Protruding tongue		+		+	
Teeth enamel hypoplasia	+				
Short neck	+	+	+	+	
Redundant skin on the back of the neck		+		+	
Pectus carinatum			+	+	
Umbilical hernia			+	+	+
5th finger clinodactyly on both hands	+	+	+	+	+
Bilateral simian creases		+	+	+	
Hypoplastic terminal phalanges of the hands			+	+	
Abnormal dermatoglyphes	+	+	+	+	+
Hip dislocation	+				+
Joint hypermobility	+	+		+	
Muscular hypotonia and hypotrophy	+	+	+	+	+

Patient 1 (IV-1)

This patient, a female, was born at term of a normal pregnancy. She is second cousin of patient 2 and 3. Her birth weight was 2,300 g. Failure to thrive and severe developmental delay were evident from early on. At 2 years, when admitted to our hospital, her height was 76 cm (<3rd centile), her weight was 7,600 g (<3rd centile), and her head circumference was 43.5 cm (<3rd centile). Her facies was characterized by mid-face hypoplasia, upslanting palpebral fissures, bilateral epicanthal folds, blue sclerae, Brushfiled spots, short nose, micrognathia, and low-set ears (Fig. 2c). Teeth enamel was hypoplastic. Short neck, distal axial triradius and 5th finger clinodactyly of both hands, hip dislocation, and joint hypermobility were also present. Neurologic examination showed severe developmental delay, muscular hypotonia, and hypotrophy. Brain MRI and cardiac evaluation, including 2D Echocardiography-color Doppler, did not show anomalies. Brain evoked visual potentials (BEVP) revealed findings compatible with a reduced visual acuity. A vesicoureteral reflux of grade II was diagnosed.

Patient 2 (IV-2)

This patient, a female, was born at term of a pregnancy characterized by spotting during the first trimester and oligohydramnios. Her birthweight was 2,300 g; severe developmental delay and failure to thrive were observed from early on. After 2 months of age she suffered several episodes of febrile seizures. At 4 years, when first admitted to our hospital, her height was 80 cm (<3rd centile), her weight was 8,250 g (<3rd centile), and her head circumference was 46 cm (<3rd centile). Her face was characterized by mid-face hypoplasia, upslanting palpebral fissures, bilateral epicanthal folds, blue sclerae, short nose, micrognathia, low-set ears, and protruding tongue (Fig. 2a). Short neck, bilateral simian creases, and distal axial triradiis were also present. Both hands showed 5th finger clinodactyly, joint laxity, and redundant skin on the back of the neck. Dermatoglyphics on right were D1-3 Lu, D4-5 W and on left D1-2 Lu, D3-4 W, D5 Lu. On trunk there was persistence of subcutaneous venous reticulum and a large mongolian spot on the back. A generalized joint laxity was also noticeable. Feet showed crowding of toes with the 3rd toe underlying the 2nd and 4th, dorsal position of the 5th toe, marked clinodactyly of the 4th toe, and microonichy. Neurologic examination showed severe developmental delay with muscular hypotrophy and hypotonia and athetoid movements. Insensitivity to pain was reported by parents and observed during venipunctures. X-ray films of the skeleton showed a thin and slender aspect of the long bones. Cardiac evaluation, including 2D-echocardiography-color Doppler, renal ultra-scan, and brain CT did not show any significant anomalies. At 5 9/12 years, her height was 84 cm (<3rd centile), her weight

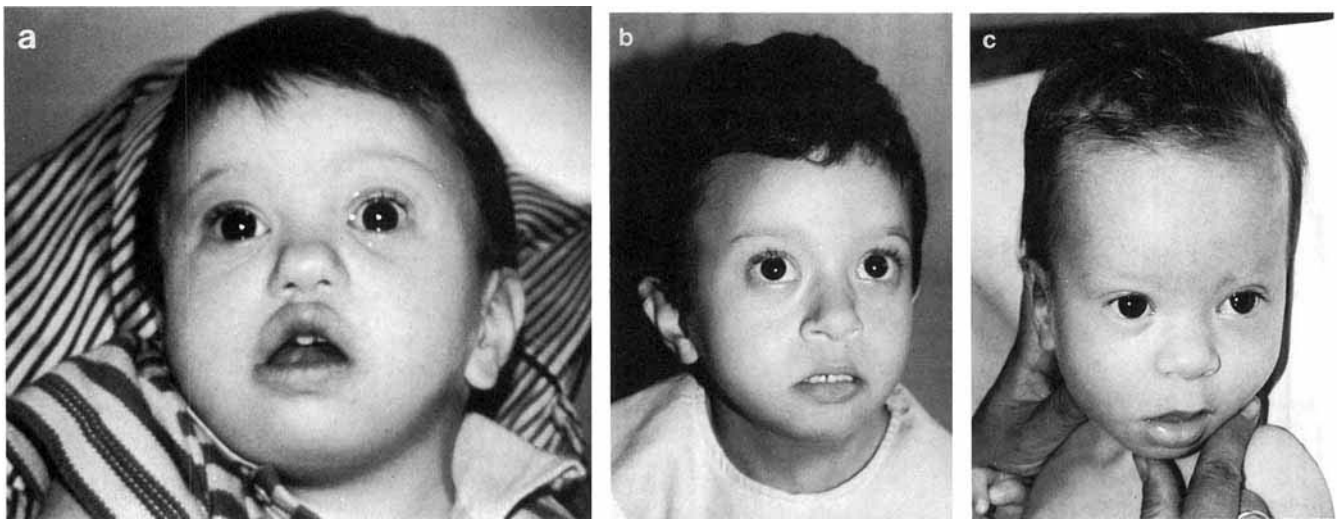

Fig. 2. **a:** Patient 1 (IV-1). **b:** Patient 2 (IV-2). **c:** Patient 3 (IV-3). Note similar physical appearance, with upslanting of the palpebral fissures, short nose, and deep set ears.

was 8,650 g (<3rd centile), and her head circumference was 46 cm (<3rd centile). At the follow-up an autistic behavior, characterized by avoidance of eye contact, absence of smile, and frequently repeated stereotypic movements, was also observed. The little girl never walked or spoke. Several standard karyotypes performed in the past have been reported as normal.

Patient 3 (IV-3)

This patient, brother of patient 2, was born at 38 weeks after a normal pregnancy. Birth weight was 2,450 g. At 7 months, when admitted to our hospital, his length was 60 cm (<3rd centile), his weight was 4,750 g (3rd centile), and his head circumference was 41 cm (<3rd centile). His facies was characterized by midface hypoplasia, upslanting palpebral fissures, bilateral epicanthal folds, blue sclerae, short nose, low-set ears, dolicocephalic skull with anteriorly placed hair whorl and frontal bossing, and short neck (Fig. 2b). The hands showed hypoplastic terminal phalanges and nails, transverse palmar crease (complete on right, incomplete on left), and hypoplasia of ridges and furrows. Dermatoglyphics on right were D1-3 Lu, D4 W, D5 Lu, and on left D1-3 Lu, D4 W, D5 Lu. Distal axial triradius and clinodactyly of the 5th finger were present on both hands. Feet showed crowding of toes and clinodactyly of 4th toe. Chest was relatively narrow with pectus carinatum, and the areolae were hyperpigmentated. There was a small umbilical hernia and a large mongolian spot on the back. Genitalia were male with descended testes. Heart sounded normal. Neurologic examination demonstrated severe developmental delay with muscular hypotrophy and hypotonia, and insensitivity to pain. At 20 months of age his length was 68 cm (<3rd centile), his weight was 6,290 g (<3rd centile), and his head circumference was 44 cm (<3rd centile). Cardiac evaluation, including 2D-Echocardiography-color Doppler, was normal. At follow-up, neurologic examination confirmed the severe developmental delay and showed a mild degree of autistic behaviour.

MATERIALS AND METHODS

Cell Cultures and Cytogenetic Analysis

Lymphoblastoid cell lines prepared from the patients were maintained in RPMI 1640 medium (Gibco) with 10% fetal bovine serum and antibiotics. Chromosomes were prepared and RHG banded according to Dutrillaux and Lejeune [1971]. Chromosome banding was produced using a standard RHG banding. High resolution analysis was performed according to Dutrillaux et al. [1981].

Construction and Characterization of Somatic Cell Hybrids

Polyethylene glycol fusions were performed between lymphoblastoid cell line from patient 3 and the hamster lung cell line FJK88 [Fuscoe et al., 1983]. Cell hybrids retaining the normal and the derivative chromosome 4 were expanded in T-75 flask and used for DNA analysis.

DNA Samples

Peripheral blood lymphocytes from the siblings and their mother were transformed by Epstein-Barr virus and lymphoblastoid cell lines were established. DNAs were extracted from peripheral blood leukocytes and/or cell lines of the parents and siblings and used for Southern blot analysis and PCR amplification.

PCR Analysis of Single Copy DNA Sequences and Dinucleotide Repeat Polymorphisms

The following chromosome 21q microsatellite markers were used for genotyping of the family members: D21S226, D21S212, D21S213, D21S211, D21S167, D21S156, D21S171, APPivs1, IFNAR, and HMG14. For chromosome 4p analysis, oligonucleotides corresponding to D4S43 and D4S115 loci were used.

Oligonucleotides for the D4S111 locus were designed on the basis of the sequence of the α-iduronidase (IDUA) gene [Scott et al., 1992]. They correspond to sequences located in introns 1 and 3, respectively: 5′cctcaggacacaccgtggaactcc and 5′gggagtcactgaggcgagattcacc.

The standard PCR reaction (25 μl) contained 500 ng of genomic DNA, 200 μM dNTPs, 50 mM KCl, 10 mM TRIS (pH 8.4), 0.2 mM BSA, 1.5 mM $MgCl_2$, 1 unit of Taq polymerase, and 200 ng of each primer, one of which was end-labeled with ($\gamma^{35}S$)-ATP using T4 polynucleotide kinase (Boehringer), when necessary. PCR amplification consisted of an initial denaturation at 95°C for 4 min and 32 cycles of denaturation at 95°C for 45 sec, annealing at 50° to 60°C (according to the primer set) for 45 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 8 min. Aliquots (4–10 μl) of PCR products were mixed with 4 μl formamide stop solution (USB), heated to 92°C for 2 min, and electrophoresed onto a 6% polyacrylamide sequencing gel at 35 W for 2–4 hrs.

FISH Analysis

The following probes from chromosome 21q21.3-qter and 4p16.3 were used for FISH analysis. From chromosome 21 are cosmid ICRFC102F10130, containing locus D21S171, and YAC 152F7 (kindly provided by D. Nizetic, ICRF, London). From chromosome 4p were the Wolf-Hirschhorn cosmid DNA probe (D4S96) and a chromosome 4 α-satellite probe (Oncor). DNA sequences of YAC152F7 were amplified by Alu-PCR using the Alu primers 517, TC-65, 278, Alu 5, and Alu 32, either alone or in combination with one or the other yeast vector primers 1089 and 1091. PCR conditions for each set of primers were as previously described [Nelson et al., 1989]. Equal aliquots of the PCR products were mixed, precipitated with ethanol and ammonium acetate, and used for labeling. Purified cosmid DNA and PCR-products were labeled with biotin 11-dUTP by nick translation using a commercially available kit (Oncor). Biotinylated probes (100 ng) were preannealed with 10 μg human placental DNA and then applied to metaphase chromosomes. Slides were incubated at 37°C for ~16 hr. After posthybridization washing, amplification, and detection were carried out using the Oncor kit and the conditions recommended by the supplier, except that the use of blocking reagents was omitted.

RESULTS

Cytogenetics

Chromosome analysis in the mother (III-2) of the two sibs (IV-2 and IV-3) and in the father (III-1) of the cousin (IV-1) demonstrated a balanced translocation (4;21)(p16.3;q22.1) leading to monosomy for the 4p telomeric region and partial trisomy of 21q (Fig. 1) in the affected offspring. Patient 1 inherited the unbalanced translocation from the father (III-1, while patients 2 and 3 inherited it from the mother (III-2). Chromosome analysis showed the same balanced translocation in other members of the pedigree (II-1, II-2).

Molecular Data

Study of the chromosome 4 monosomic region. The D4S43 STR pattern of inheritance was successfully used to distinguish between hybrids retaining either the maternal derivative chromosome 4 or the paternal normal chromosome 4. Patient 1 was heterozygous for the D4S43 STR and two cell hybrid clones, GA9 and GA11, enabled the amplification of one allele only, maternal and paternal, respectively.

The two somatic cell hybrid lines, GA9 and GA11, were analyzed with oligonucleotides derived from D4S43, D4S115, and IDUA loci. While GA11, containing the normal paternal chromosome 4, displayed a normal pattern of amplification, GA9 showed only one band related with D4S43, being D4S115 and IDUA loci deleted (Fig. 3a), thus suggesting the location of the breakpoint between D4S43 and D4S115.

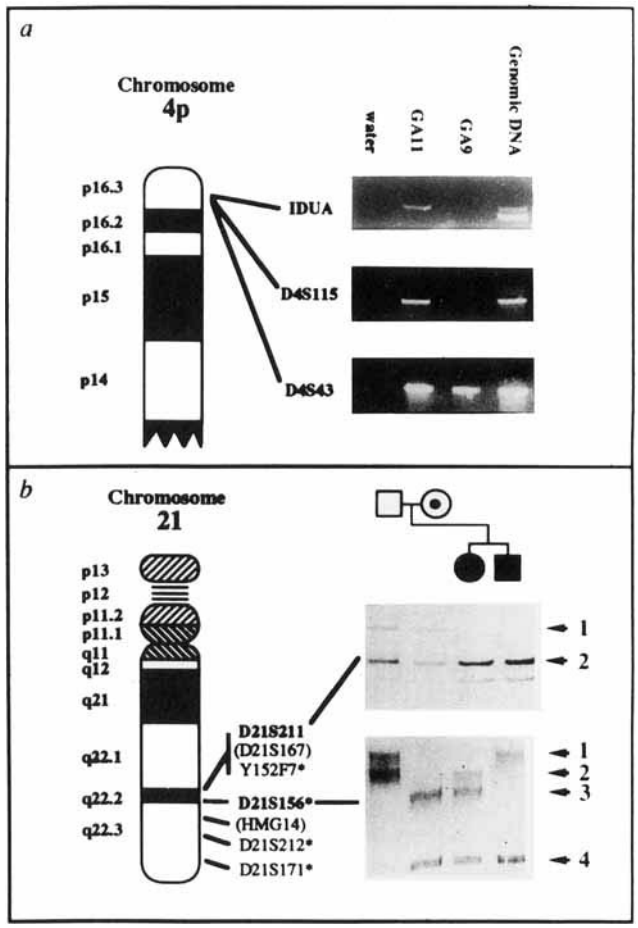

Fig. 3. **a:** PCR analysis with markers IDUA, D4S115 and D4S43 of somatic cell hybrids GA11 and GA9, retaining the normal and deleted chromosome 4, respectively. Monosomy at the IDUA and D4S115 loci is demonstrated by lack of amplification at these loci. **b:** Microsatellite analysis of 21q markers in patients 2 and 3 and their parents. At D21S211, the most centromeric locus, both sibs show only one of the two different maternal alleles, excluding trisomy at this locus. Trisomy at the D21S156 locus is demonstrated by the presence of three alleles (see text). Asterisks indicate other markers resulted to be trisomic, while some of the uninformative probes are reported in parenthesis.

Characterization of the trisomic 21 region. The allelic content of ten dinucleotide repeat polymorphic markers, mapping to different regions of chromosome 21, was analyzed in the siblings (IV-2 and IV-3) and their carrier parents. Trisomy for the daughter at the loci D21S156 (Fig. 3b) and D21S212 (not shown) is demonstrated by the presence of three different alleles, two of which are of maternal origin. At the same loci her brother showed only one of the two different types of maternal alleles. However, visual inspection of the intensity of the allelic fragments suggests dosage effect and inheritance of two doses of one of the maternal alleles due to a recombination event at maternal meiosis between the normal 21 and the t(4;21) chromosome. At the more proximal loci APPivs1 and D21S211 (Fig. 3b) both sibs showed only one of the two different maternal alleles, suggesting disomy at these loci. All other markers were uninformative (data not shown). Thus, the data obtained by microsatellite analysis suggest that the breakpoint is located between the markers D21S211 and D21S156.

FISH Studies

To determine more precisely the proximal boundary of the trisomic 21 region FISH analysis was performed using Alu-PCR products of the YAC 152F7 mapping proximal to D21S55 [Chumakov et al., 1992] in 21q22.2. Label was found on the normal chromosome 21 and the derivative chromosome 4 of the mother (Fig. 4A) and her daughter. This finding locates the breakpoint within the proximal part of the Down syndrome critical region (DSCR), a chromosomal segment of approximately 2 Mb flanked by D21S211 and D21S267 [Dufresne-Zacharia et al., 1994].

To establish if the trisomic 21 region extends to 21qter, FISH was also performed on chromosomes from patient 2 and her mother, using as probe a cosmid containing locus D21S171 from the telomeric region. Figure 4b shows the label on the normal chromosome 21 and on the distal short arm of the derivative chromosome 4 of the patient, suggesting that the translocated region extends to 21qter.

For a detailed definition of the 4p monosomic segment, we performed FISH studies on the chromosomes of patient 2 and her mother using the Oncor Wolf-Hirschhorn cosmid probe at locus D4S96. Label was detected on the normal and the derivative chromosomes 4 in the affected daughter (Fig. 4c). Her mother's chromosomes showed label on the normal and the derivative chromosomes 4 as well as on the derivative chromosome 21 (Fig. 4d). The consistent finding of three labels in 18/18 maternal metaphases suggests that the translocation breakpoint lies within the region identified by this cosmid.

DISCUSSION

We have observed three children from the same family affected by an aneuploidy syndrome characterized by severe growth and developmental delay and mild physical anomalies. Cytogenetic investigation demonstrated the existence of the parental translocation (4;21)(p16.3;q22.1). The unbalanced segregation of this translocation caused a double segmental aneuploidy, partial 4p monosomy and partial 21q trisomy, involving the critical region of WHS and DS, respectively. The clinical picture of these patients is probably the result of a combination of DS and WHS elements. The two most striking findings are severe neurologic involvement and failure to thrive. Surprisingly, no major malformation, such as congenital heart defect, has been detected in any of these patients, despite a relative risk higher than 50% for either WHS or DS. The mild physical anomalies recall DS more than WHS. As a matter of fact, the chromosome 21 breakpoint was mapped within the proximal part of DSCR in 21q22.2, indicating that the region DSCR-telomere is trisomic in our patients. The facial and physical anomalies present in our patients are consistent with the phenotype-genotype correlations described previously for other partial trisomies 21 [Delabar et al., 1991].

As to the definition of the breakpoint on chromosome 4, it was mapped by molecular studies to a region between D4S43 and D4S115. FISH analysis indicated that the breakpoint lies within locus D4S96. In fact, a double signal is visible on both the derivative chromosome 4 and the derivative chromosome 21 of the maternal metaphases. These data suggest that only about 1.3 Mb of the telomeric region of 4p is deleted. Being aware that the present patients combine a double segmental aneuploidy, some cautious speculation may be made on the role of the deleted 4p region. Typical WHS facial traits, such as "Greek helmet" appearance, preauricular dimple or skin tag, and congenital heart defects are not present in our patients. External genitalia of patient 2 do not show anomalies such as hypospadia, frequently reported in males. In patient 1 a vesicoureteral reflux was found, but not investigated in the other patients for absence of urinary symptoms. Recently, evidence has been given that the WHSCR should be restricted to a region of approximately 2.2 Mb, distal to D4S43 and proximal to D4F26 [Zackai et al., 1994]. The finding that in the present patients only about half of that region is monosomic suggests that this part is probably not critical for major malformations (Fig. 5). However, the profound neurologic involvement and the severe failure to thrive can be hardly ascribed to the DS alone. Therefore, some genes involved in the control of both brain development and growth may reside in the 1.3 Mb telomeric segment of 4p.

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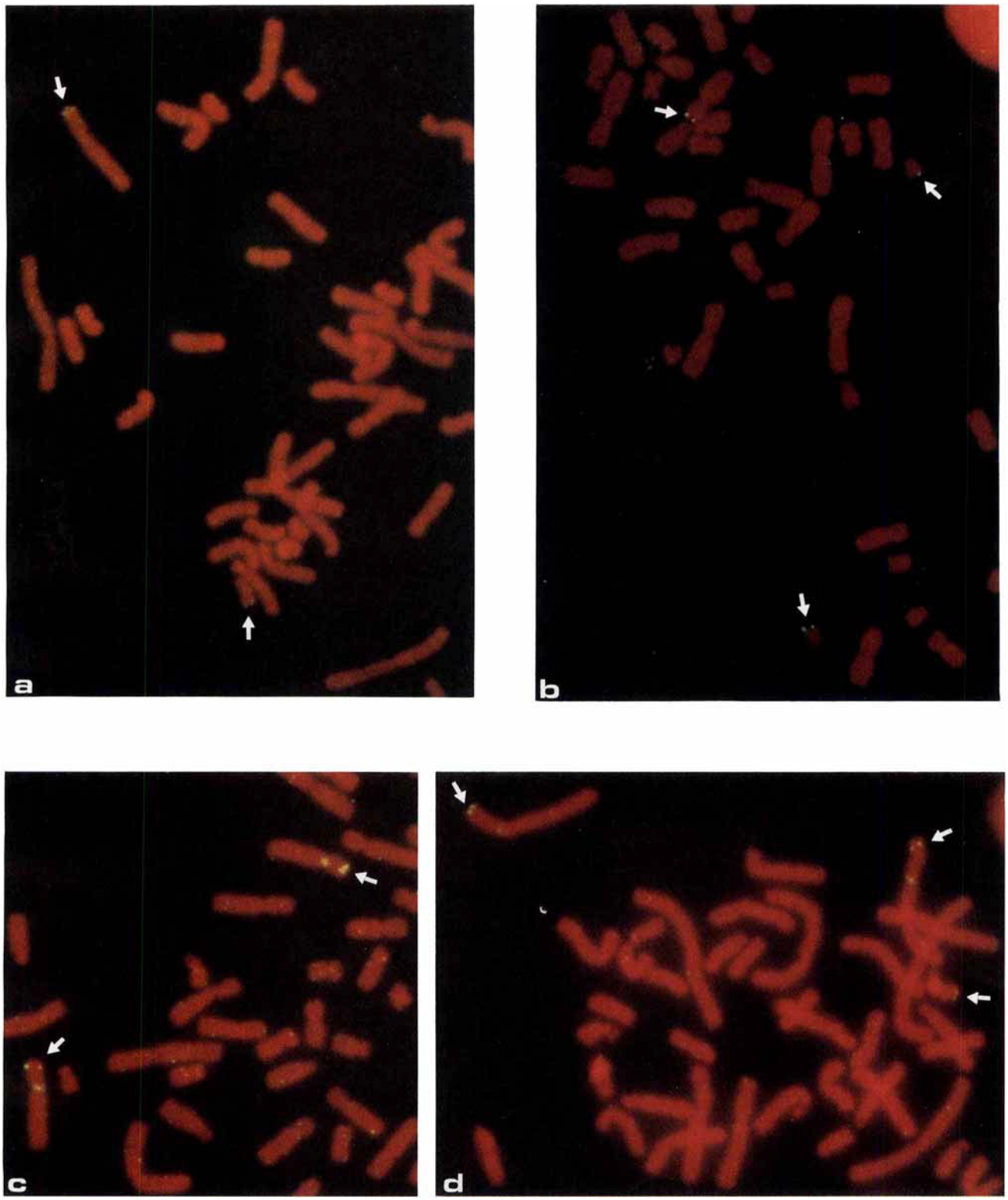

Fig. 4. FISH analysis on patient 2 (IV-2) and her mother (III-2). **a:** Hybridization of Alu-PCR products of YAC 152F7 to chromosomes of the mother of the patient. Note the hybridization signals on both the normal chromosome 21 and derivative chromosome 4. **b:** Hybridization of a cosmid, containing locus D21S171, to metaphase cells of the patient. Arrows indicate the signals on the derivative chromosomes 4 and 21 and the normal chromosome 21. **c:** Hybridization of the WH-Oncor probe (D4S96) to chromosomes of patient 2. Fluorescent signals are observed on both the normal and derivative chromosome 4. **d:** Hybridization of the WH-Oncor probe (D4S96) to metaphase cells of the mother of patient 2. Arrows indicate hybridization to both the normal and derivative chromosome 4 and to the derivative chromosome 21.

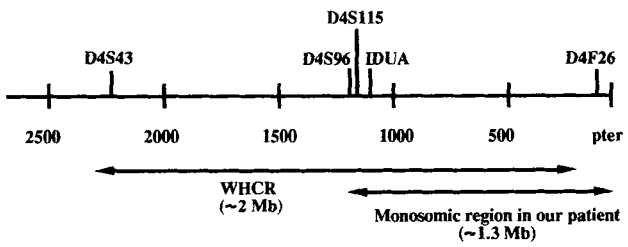

Fig. 5. The 4p region deleted in the present patients, involving approximately 1.3 Mb of the candidate Wolf-Hirschhorn critical region (WHSCR).

REFERENCES

Chumakov I, Rigault P, Guillou S, Ougen P, Billaut A, Guasconi G, Gervy P, LeGall I, Soularue P, Grinas L, Bougueleret L, Bellané-Chantelot C, Lacroix B, Barillot E, Gesnouin P, Pook S, Vaysseix G, Frelat G, Schmitz A, Sambucy JL, Bosch A, Estivill X, Weissenbach J, Vignal A, Riethman H, Cox D, Patterson D, Gardiner K, Hattori M, Sakaki Y, Ichikawa H, Ohki M, Le Paslier D, Heilig R, Antonarakis S, Cohen D (1992): Continuum of overlapping clones spanning the entire human chromosome 21q. Nature 356: 380–387.

Dallapiccola B, Mandich P, Bellone E, Selicorni A, Mokin V, Ajmar F, Novelli G (1994): Parental origin of chromosome 4p deletion in Wolf-Hirschhorn syndrome. Am J Hum Genet 47:921–924.

Delabar JM, Theophile D, Rahmani Z, Chettouh Z, Blouin JL, Prieur M, Noel B, Sinet PM (1993): Molecular mapping of twenty-four features of Down syndrome on chromosome 21. Eur J Hum Genet 1: 114–124.

Dufresne-Zacharia MC, Dahmane N, Theophile D, Orti R, Chettouh Z, Sinet PM, Delabar JM (1994): 3.6-Mb genomic and YAC physical map of the Down syndrome chromosome region on chromosome 21. Genomics 19:462–469.

Dutrillaux B, Lejeune J (1971): Sur le novelle technique d'analyse du caryotype humain. C R Acad Sci (Paris), série D 272:2638–2640.

Duttrillaux B, Viegas E, Pequignot L (1981): High resolution R and G banding on the same separation. Hum Genet 57:93–95.

Estabrooks LL, Lamb AL, Aylsworth AS, Callanan NP, Rao KW (1994): Molecular characterization of chromosome 4p deletions resulting in Wolf-Hirschhorn syndrome. J Med Genet 31:103–107.

Fuscoe JC, Fenwick RG, Ledbetter DH, Caskey CT (1983): Deletion and amplification of the HGPRT locus in Chinese hamster cells. Mol Cell Biol 3:1086–1096.

Gandelman KU, Gibson L, Meyn MS, Yang Feng TL (1992): Molecular definition of the smallest region of deletion overlap in the Wolf-Hirschhorn syndrome. Am J Hum Genet 51:571–578.

Korenberg JR, Chen XN, Schipper R, Sun Z, Gonsky R, Gerwehr S, Carpenter N, Daumer C, Dignan P, Disteche C, Graham JM Jr, Hugdins L, McGillivray B, Miyazaki K, Ogasawara N, Park JP, Pagon R, Peuschel S, Sack G, Say B, Schuffenhauer S, Soukup S, Yamanaka T (1994): Down syndrome phenotypes: The consequences of chromosomal imbalance. Proc Natl Acad Sci USA 91: 4997–5001.

Lurie IW, Lazjuk GI, Ussova YI, Presman EB, Gurevich DB (1980): The Wolf-Hirschhorn syndrome. Clin Genet 17:375–384.

Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis R, Webster TD, Ledbetter DH, Caskey CT (1989): Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. Proc Natl Acad Sci USA 86: 6686–6690.

Scott HS, Litjens T, Hopwood JJ, Morris CP (1992): PCR detection of two RFLPs in exon 1 of the α-L-iduronidase (IDUA) gene. Hum Genet 90:327.

Wilson MG, Towner JW, Coffin GS, Ebbin AJ, Siris E, Brager P (19810: Genetic and clinical studies in 13 patients with the Wolf-Hirschhorn syndrome (del(4p)). Hum Genet 59:297–307.

Zackai EH, McDonald-McGinn DM, Spinner N, Alther MR, Bengtsson U, Wasmuth JJ (1994): Classical Wolf-Hirschhorn syndrome confirmed molecularly despite normal results using commercially available probes: redefinition of critical region. Am J Hum Genet 53:A47.